PATENT
ATTORNEY DOCKET NO. 50001/002005

Certificate of Mailing: Date of Deposit: June 14, 2005

I hereby certify under 37 C.F.R. § 1.8(a) that this correspondence is being deposited with the United States Postal Service as **first class mail** with sufficient postage on the date indicated above and is addressed to Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Susan M. Cannon
Printed name of person mailing correspondence

Susan M Cannon
Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	George A Gaitanaris	Art Unit:	1636
Serial No.:	09/982,586	Examiner:	Qian, Celine
Filed:	October 17, 2001	Customer No.:	21559
Title:	VECTORS AND METHODS FOR THE MUTAGENESIS OF MAMMALIAN GENES		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. GEORGE A. GAITANARIS

1. I am an inventor on the above-captioned patent application.

2. I have read the above-referenced patent application as well as the Office Action mailed December 14, 2004 ("the Office Action").

3. The mice of the invention have two transgenes. The first transgene includes a regulatory gene encoding a regulatory protein. In the mice of the invention, this first transgene has integrated into an endogenous gene of a mouse such that the endogenous gene is mutated and the regulatory gene is positioned for expression under control of the promoter of the endogenous gene. The second transgene includes a gene operably linked to a regulatory sequence regulated by the regulatory protein encoded by the first transgene. This second transgene is

integrated into the genome of the mouse such that expression of the operably linked gene is regulated by the regulatory protein.

4. In the Office Action, the Office contends that the production of mice in which the first and second transgenes are expressed at sufficient levels is not enabled by the specification because of the influence of positional effects and the strength of the promoter of the endogenous gene. As is demonstrated by the prior art and post-filing studies, positional effects, while present, do not hinder one's ability to make the claimed mice.

5. As of the priority date of the present application, researchers had produced mice expressing a transgene positioned for expression under control of the promoter of an endogenous gene (corresponding to the "first transgene" in the claims). For example, in 1991, Friedrich and Soriano described the production of more than two dozen different mouse lines containing transgenes expressed under the control of a promoter of an endogenous gene (Genes Dev. 5:1513-1523, 1991; Exhibit A). In 75% of these mouse lines, the protein encoded by the transgene could be detected (page 1517, left col.). Moreover, many of the lines were demonstrated to be mutagenic; nine of twenty-four lines crossed to homozygosity were embryonic lethal, indicating a mutation in a gene required for embryonic development. Presumably many of the other transgene insertions were similarly mutagenic, but their phenotypes less overt. In another study, Wurst et al. (Genetics 139:889-899, 1995; Exhibit B) demonstrated that this method of expressing transgenes from endogenous promoters could be performed on a large-scale basis. Wurst produced more than 300 mouse embryonic stem (ES) cell clones containing integrations of a *lacZ* transgene. When these clones were used to make 8.5-day chimeric mouse embryos, "approximately one third of the clones showed widespread *lacZ* expression" and "[t]hirty-five...exhibited tissue specific or spatially-restricted expression patterns..." (page 895, right col.). Based on the

teachings of Friedrich and Soriano, one would expect that many of Wurst's mouse lines would also contain a mutation. Thus, as of the priority date of the present application, the ability to make mice expressing a transgene positioned for expression under the control of the promoter of an endogenous gene was well within the skill of one in the field of mouse genetics. As evidenced by the prior art, transgene insertion frequently resulted in expression of the protein encoded by the transgene and mutation of the endogenous gene. The methods used to make these mice were routine, and a person in the field embarking on production of such a transgenic mouse would reasonably expect to succeed.

6. As of the priority date of the present application, researchers had also produced mice expressing a gene operably linked to a regulatory sequence that itself is regulated by a regulatory protein encoded by another transgene (corresponding to the "second transgene" of the present claims). For example, Furth et al. (Proc. Natl. Acad Sci. USA 91:9302-9306, 1994; Exhibit C) produced mice carrying (i) a luciferase or β-galactosidase reporter gene fused to seven *tet* operator sequences; and (ii) a transgene encoding a tetracycline-controlled transactivator fusion protein (tTA) composed of the *tet* repressor and the VP16 activation domain under the control of the hCMV IE1 promoter/enhancer (page 9302, right col.). In these mice, the reporter gene was expressed in the absence of tetracycline, and repressed upon the addition of tetracycline (Tables 1 and 2). Positional effects (i.e., gene expression due to the insertion locus) were examined by determining reporter gene expression levels in the presence of tetracycline. The authors reported only "[t]he sporadic occurrence of measurable levels of luciferase in some mice" (page 9305, right col.). This same general methodology has been employed on numerous occasions, and a selected few exemplary publications are attached as Exhibits D-G. It is clear from the abundance of examples in the literature that the production of mice expressing a gene operably linked to a regulatory sequence that itself is regulated by a regulatory protein

encoded by another transgene was well within the skill of a mouse biologist as of the filing date of the present application.

7. Moreover, while the potential problem, in some mice, of positional effects in inducible gene expression systems had been recognized, possible solutions had also been recognized. For example, Shockett and Schatz (Proc. Natl. Acad. Sci. 93: 5173-5176, 1996; Exhibit H) suggest that "[i]ntegration site-specific effects...might be overcome by surrounding individual transcription units with matrix attachment regions, shown previously to insulate stably integrated vectors and transgenes from effects mediated by cis regulatory elements adjacent to their sites of integration [citations omitted]." Insulators have since been shown to reduce positional effects in inducible gene expression systems (Exhibits I and J). Regardless, the examples provided above demonstrate that the positional effects do not hinder one's ability to successfully make the transgenic mice. Rather, positional effects become relevant when the complete lack of basal activity is required for a special purpose.

8. As mice having either the first or second transgene are readily produced using techniques known as of the priority date of the present application, the only remaining issue is whether mice having both transgenes expressed at sufficient levels can be produced. As is discussed in the specification, one desirable way to make mice having two transgenes is to cross a mouse having the first transgene with a mouse having the second transgene. Subsequent to the filing of the above-referenced patent application, researchers in my laboratory have used this method and made exemplary mice of the present invention.

9. In one example, we produced two lines of mice. The first line had a mutation in an endogenous gene (ApoE) and expressed a regulatory protein (a tetracycline repressor fused to a VP16 transcriptional activator) regulated by the

ApoE promoter. The second line had an ApoE transgene integrated into the genome and operably linked to a regulatory sequence that, in the presence of tetracycline, was expressed under the control of the regulatory protein. The transgene/regulatory sequence complex was flanked by the chicken ß-globin locus control region (Chung, J. H., Whiteley, M. & Felsenfeld, G. (1993) Cell 74, 505-14). This control region has been reported to insulate the flanked gene from the enhancer activity of the surrounding region. To produce the claimed mice, we crossed the two mouse lines to produce mice having both transgenes (termed "ApoE IKO mice").

10. To characterize the ApoE IKO mice, we examined both heterozygous and homozygous animals. The results are shown in Exhibit K. Mice were fed with doxycycline (Dox) in their normal chow food to induce tetracycline dependent gene expression of the transgenic ApoE gene. Heterozygous mice had one of the two endogenous ApoE (endoApoE) alleles inactivated and also contained the ApoE under the control of the tetOP promoter, producing the inducible-ApoE mRNA. Using RT-PCR we determined the levels of endoApoE, inducible-ApoE, and hybrid endoApoE-rtTA transcripts in the liver, the major site of normal ApoE production, in the presence or absence of Dox (Exhibit K, left panels). As expected, expression of endoApoE and rtTA were independent of Dox. However, expression of inducible-ApoE was strictly dependent on Dox – it was undetectable in its absence and significantly expressed in its presence, indicating the high degree of ApoE regulation achieved in the mice (left panels). Next, we carried out a phenotypic analysis of homozygous ApoE IKO mice, i.e. mice having both endogenous ApoE alleles inactivated and carrying inducible-ApoE in the genome. In these mice, ApoE protein can only be produced from the inducuble-ApoE gene and this production is under the control of Dox. Absence of ApoE protein is expected to lead to hypercholesterolemia. We analyzed blood plasma cholesterol levels in these mice in the absence and presence of Dox. As controls we used

wild-type (WT) and ApoE KO mice. In the ApoE KO mice, both endogenous ApoE alleles were inactivated by the retroviral insertion and no inducible-ApoE allele was present in these animals. As shown in Exhibit K, the IKO mice had high cholesterol levels in the absence of Dox, comparable to that of the ApoE KO mice, whereas, after the mice were fed Dox food, their cholesterol level came down to normal range, demonstrating that inducible expression of ApoE can lead to correction of the KO phenotype (Exhibit K, right panel). When Dox food was withdrawn, the cholesterol level in the IKO mice went back up again (Exhibit K, right panel). These on/off switches occurred rapidly (within one week after Dox administration and withdrawal).

11. As is shown by the foregoing results, the ApoE IKO mice (i) express the first transgene from an endogenous promoter; (ii) express the second transgene in a manner that is regulated by the regulatory protein encoded by the first transgene; and (iii) exhibit a phenotype that can be altered by regulating the expression of the second transgene. That the second transgene's expression is regulated by the regulatory protein demonstrates that the first transgene is expressed at a sufficient level to regulate the second transgene. The ability to alter a mouse's phenotype by altering the expression of the second transgene demonstrates that the second transgene is similarly expressed at a sufficient level.

12. In sum, the numerous prior examples of successful production of mice carrying one of the two transgenes combined with the general knowledge in the field demonstrates that one skilled in mouse biology could make mice carrying both transgenes without undue experimentation, and that positional effects, while present, do not preclude the successful practicing of the invention, a conclusion further supported by applicant's own successful reduction to practice of the invention.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date June 13, 2005

George A. Gaitanaris, Ph.D.